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Sensitivity of the Neotropical Teleost *Odonthestes bonariensis* (Pisces, Atherinidae) to Chromium(VI), Copper(II), and Cadmium(II)

P. Carriquiriborde, A. Ronco

Environmental Research Center, Faculty of Sciences, National University of La Plata and National Research National Council, 47 y 115, (1900)-La Plata, Argentina

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Fish have been historically used to assess biological effects of xenobiotics in aquatic environments. Although most studies have been conducted using species of the Holartic Region, the assessment of the sensitivity to toxicants on Neotropical species is recent (Ronco *et al.* 2000). The atherinid teleost *Odonthestes bonariensis* is a widespread and characteristic fish species from the meridional sector of the 'del Plata' basin. It inhabits shallow ponds, rivers and estuaries with moderately hard to hard waters with variable conductivity, from fresh to brackish (Conzono and Claverie 1990; Villar and Bonetto 2000). This oviparous and planktonic fish has an important economic value due to both sport and commercial fishing (Bonetto and Castello 1985) and has been introduced in many other geographical regions from Bolivia and Chile to Japan (Welcome 1988). In spite of the extensive literature available on its biology (Lopez *et al.* 1991) and its phylogenetic and ecological relationship to *Menidia beryllina* a species widely employed in bioassays, the effect of environmental contaminants on *O. bonariensis* has been poorly studied (Menone *et al.* 2000; Carriquiriborde *et al.* 2000). A recent publication reports on the sensitivity to toxicants of *Odonthestes regia*, a marine species, from the Pacific Ocean (Silva *et al.* 2001).

The present study reports on the sensitivity of *O. bonariensis* to salts of three toxic metals of environmental significance - Cr(VI), Cu(II) and Cd(II) -, in laboratory acute toxicity tests, in relation with the origin and age of the organisms, time of exposure and the water chemistry. Results will be useful for future risk assessment studies and planning strategies in environmental programs of the region.

MATERIALS AND METHODS

The fish were obtained from two different sources. One group of recently hatched larvae from the aquaculture station of the Buenos Aires Province Ministry of Production (Chascomús, Buenos Aires, Argentina) came from eggs collected in the Gómez lagoon (34° 35' S, 61° 7' W, Junín, Buenos Aires). The other group was obtained by field fertilization of eggs from the Lobos lagoon (35° 17' S, 59° 7' W, Lobos, Buenos Aires) and incubated in our laboratory. All fish were raised

Correspondence to: A. E. Ronco

in the laboratory in twelve 60 L culture chambers in a 3,500 L aquarium with a closed water circulation system and fed with 24 hr nauplii of *Artemia* sp *ad libitum*. Feces and organic debris were siphoned out daily. Water used for culturing organisms was La Plata tap water (conductivity 1.0 mS cm^{-1}, hardness 215 mg $CaCO_3$ L^{-1}, alkalinity 183 mg $CaCO_3$ L^{-1}, pH 7.4), filtered through activated carbon for organic and chlorine removal (Cole Palmer, cartridge P-99260-08). Chemical parameters were determined in the laboratory according to standardized methods (APHA 1998). Water temperature was maintained at 22 ± 1 °C and dissolved oxygen at a concentration level of 7.0 mg L^{-1} or greater. Photoperiod was set to 16 L: 8 D.

Toxicity tests were carried out every spring season (species reproductive period) from 1997 through 2000 under the same conditions as the ones used for culturing. Acute assays were done using 2 (9.1 ± 1.6 mm, n= 10) and 4 (12,8 ± 1.3 mm, n= 10) week old *O. bonariensis* fry from the two mentioned sources. Tests were carried in 1 L polypropylene jars filled with 0.5 L of testing water, using 10 organisms per jar, 3 replications per concentration, 5 toxicant concentrations (as metal ion) - Cr(VI) from 0.5 to 80 mg L^{-1}; Cu(II) from 0.01 to 1 mg L^{-1}; Cd(II) from 0.005 to 0.5 mg L^{-1} - and controls. The metal concentration in each stock solution was verified by atomic absorption spectrophotometry (APHA 1998). Toxicity test type was static with water renewal every 24 hr. Test chambers were rinsed with metal dilutions before testing. Fish were fed with 0.5 mL of concentrated 24 hr *Artemia* sp nauplii in test water, 2 hr before the 48 hr water renewal. Tests were conducted over 96 hr, reading mortality every 24 hr. Fish were considered dead when opercular movements ceased. A test was taken as valid when mortality in control population was less than 10%. Tests using moderately hard water were done with two week organisms from Lobos for Cu(II) and Cd(II). Results were analyzed using the PROBIT parametrical statistic (US EPA software version 1.5) to estimate LC_{50}, slope and *y* intercept of transformed dose response function at 24, 48 and 96 hr exposure (US EPA 1993). Statistical significant differences (α=0.05) of LC_{50} between treatments were assessed according to Finney (1971).

Toxicant dilutions were prepared from stock solutions of $K_2Cr_2O_7$ (Anedra) $CuSO_4 \times 5H_2O$ (Anedra) and $CdSO_4$ (Carlo Erba) in two different types of water: hard water from the tap (same as used for culturing) and moderately hard (USEPA 1993) prepared from commercial soft drinking water (Chispal®) adding $NaHCO_3$, $CaSO_4$ and $MgSO_4$. All reagents used were analytical grade.

RESULTS AND DISCUSSION

The general behavior of the sensitivity range of variation for *O. bonariensis* to Cr(VI) according to the LC_{50} values shows an increase of one order of magnitude between 24 and 96 hr exposure (Table 1). The response to the metal at 48 hr exposure shows lower variation between different treatments (age and origin of organisms). Differences in the response to Cr(VI) with the age of the organisms was found significant only after 96 hr exposure, showing a slightly higher sensitivity for 2-week old organisms. The origin of the organisms did not affect the sensitivity to Cr(VI).

The observed transformed dose response functions (Fig. 1) indicate higher variability in slope between treatments for 24 hr exposure, while after 48 hr the slopes show a similar trend.

Table 1. Sensitivity of *O.bonariensis* to Cr(VI) after 24, 48 and 96 hr exposure, in relation with the origin and age of the organism.

	24h		48h		96h	
Test	LC_{50} (mg L^{-1})	Confidence Interval (95%)	LC_{50} (mg L^{-1})	Confidence Interval (95%)	LC_{50} (mg L^{-1})	Confidence Interval (95%)
Lo2H	36.47 [a]	29.06-42.95	19.45 [a]	11.74-24.65	1.46 [a]	0.66-2.65
Lo4H	35.05 [a,b]	29.47-39.82	13.10 [a]	5.91-18.99	4.53 [b]	1.58-7.52
Ju4H	20.84 [b]	14.87-32.11	14.40 [a]	10.90-19.95	8.20 [b]	5.29-11.69

Values followed by the same letter are not significantly different (p>0.05)
Lo: Lobos, Ju: Junín; 2 and 4: age in weeks; H: hard water

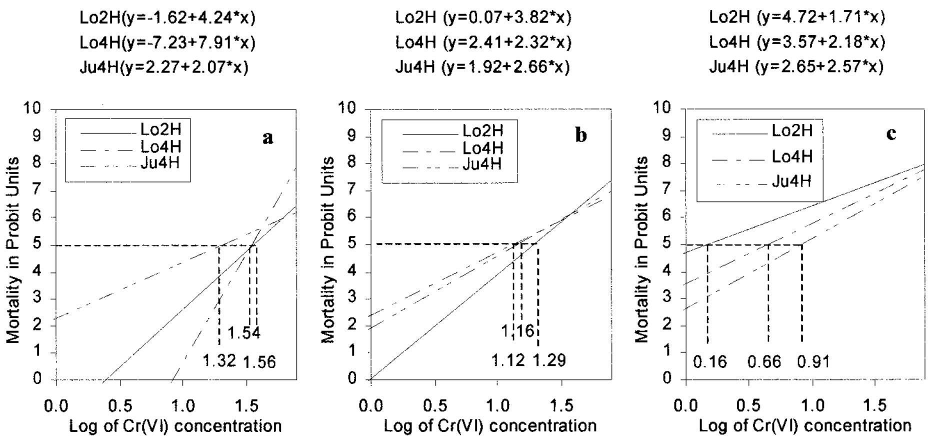

Figure 1. Ajusted Probit and predicted regression lines showing the response behavior of *O. bonariensis* to Cr(VI) in relation with the origin and age of organism after 24 (a), 48 (b) and 96 hs (c) exposure. Lo: Lobos, Ju: Junín; 2 and 4: age in weeks; H: hard water.

The average sensitivity range of variation of the species to Cu(II) expressed as LC_{50} increases only three times between 24 and 96 hr exposure (Table 2). The highest sensitivity was observed after 96 hr exposure with organisms from Junín 2-week old. All the studied factors seem to affect the sensitivity of *O. bonariensis* to Cu(II). Moderately hard water increases the sensitivity of response in all tested conditions. Organisms from Junín appear to be more sensitive than those from

Lobos. Sensitivity decreases with the age of fish. A compensation of the effect of age and origin can be observed in the comparison between 4-week old Junín and 2-week old Lobos fish.

In all cases transformed dose response functions (Fig. 2) show a similar behavior, with the exception of 2-week old organisms from Junín in hard water, where a change of slope was detected.

Table 2. Sensitivity of *O.bonariensis* to Cu(II) after 24, 48 and 96 hs exposure, in relation with the origin and age of organism and type of water.

	24h		48h		96h	
Test	LC_{50} (mg L^{-1})	Confidence Interval (95%)	LC_{50} (mg L^{-1})	Confidence Interval (95%)	LC_{50} (mg L^{-1})	Confidence Interval (95%)
Lo2MH	0.225 [a]	0.139-0.328	0.090 [a]	0.054-0.134	---	---
Lo2H	0.608 [b]	0.456-0.811	0.383 [b]	0.265-0.675	---	---
Ju2H	---	---	0.123 [a]	0.091-0.159	0.080 [a]	0.063-0.098
Ju4H	0.436 [c]	0.306-0.800	0.374 [b]	0.252-0.628	0.218 [b]	0.148-0.297

Values followed by the same letter are not significantly different (p>0.05)
Lo: Lobos, Ju: Junín; 2 and 4: age in weeks; H: hard water, MH: moderately hard water, ---: LC_{50} estimate could not be calculated, less than two observed proportion mortalities were detected between zero and one.

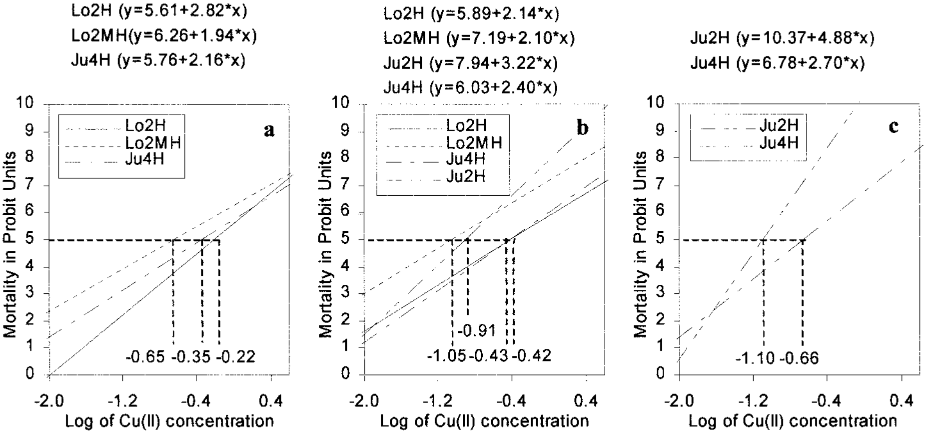

Figure 2. Plots of adjusted Probit and predicted regression lines showing the response behavior of O. bonariensis to Cu(II) in relation with the origin and age of organism and type of water, after 24 (a), 48 (b) and 96 hs (c) of exposure. Lo: Lobos, Ju: Junín; 2 and 4: age in weeks; H and MH: hard and moderately hard water, respectively.

The sensitivity range of variation with time to Cd(II) varies 5 times between 24 and 96 hr exposure (Table 3). The lowest LC_{50} found was 0.015 mg L^{-1} of Cd(II) with organisms from Junín 2-week old. Even though the proportion of experimental data with this metal is lower, we can observe that water hardness seems to be the dominant factor over age and origin in the metal sensitivity. Organisms in moderately hard water always appear to be more sensitive. In addition, in a similar way as for Cu(II), it was observed that organisms from Junín appear to be more sensitive and the age of fish decreases it.

No highly relevant differences between the studied conditions could be observed in transformed dose response plots (Fig. 3).

Table 3. Sensitivity of *O.bonariensis* to Cd(II) after 24, 48 and 96 hs exposure, in relation with the origin and age of organism and type of water.

	24h		48h		96h	
Test	LC_{50} (mg L^{-1})	Confidence Interval (95%)	LC_{50} (mg L^{-1})	Confidence Interval (95%)	LC_{50} (mg L^{-1})	Confidence Interval (95%)
Lo2H I	0.093 [a]	0.061-0.653	---	---	---	---
Lo2H II	---	---	0.101 [a]	0.073-0.141	---	---
Lo4MH	0.035 [b]	0.012-0.055	---	---	---	---
Ju2H	---	---	0.015 [b]	0.009-0.021	---	---
Ju4H	0.292 [c]	0.200-0.513	0.053 [c]	0.039-0.068	0.036	0.006-0.049

Values followed by the same letter are not significantly different (p>0.05)
Lo: Lobos, Ju: Junín; 2 and 4: age in weeks; H: hard water, MH: moderately hard water, ---: LC_{50} estimate could not be calculated, less than two observed proportion mortalities were detected between zero and one.

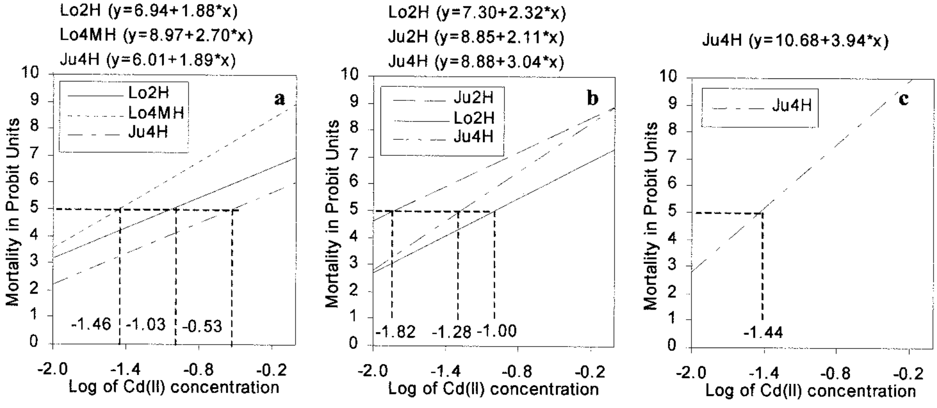

Figure 3. Plots of adjusted Probit and predicted regression lines showing the response behavior of *O. bonariensis* to Cd(II) in relation with the origin and age of organism and type of water, after 24 (a), 48 (b) and 96 hs (c) of exposure. Lo: Lobos, Ju: Junín; 2 and 4: age in weeks; H and MH: hard and moderately hard water, respectively.

A comparison of the results of acute toxicity tests with the three tested metals shows the following trend of sensitivity for *O. bonariensis*: Cd(II)>Cu(II)>Cr(VI). Copper(II) and Cd(II) toxicity significantly decreases in hard water in agreement with published data for other species (Welsh *et al.* 1996, USEPA 2000). In general, the tendency detected with factors such as age and origin indicates that younger organisms and Junín source appear to determine higher sensitiveness. When considering the combined effect of origin and age, results indicate an additive behavior (e.g., effect of Cu(II) at 48 hr on 2 weeks old organisms from Lobos is equivalent to the one observed on 4-week old organisms from Junín).

Measured mean sensitivity of *O. bonariensis* to Cr(VI) in hard water (96 hr LC_{50} 5.4 mg L^{-1}) is at least one order of magnitude higher than that reported for other fresh water fish species (Rehwoldt *et al.* 1972, Van Leeuwen 1990, Dorn *et al.* 1993). When compared to the marine species from the same genus *O. regia* (Silva *et al.* 2001), *O. bonariensis* proves to be twice more sensitive. On the other hand, the medium Cu(II) 96 hr LC_{50} value determined for *O. bonariensis* of 0.15 mg L^{-1} is equivalent to that published for *M. berylina* -fresh water- (USEPA 1993) and *M. menidia* -salt water- (Anderson *et al.* 1991), but almost one order of magnitude less sensitive than *O. regia* (Silva *et al.* 2001). In comparison with another Neotropical species, we can observe that *O.bonariensis* at 48 hr exposure to Cu(II) in moderately hard water (LC_{50} 0.090 mg L^{-1}) is significantly more sensitive than adult organisms of *Cnesterodon descenmaculatus* in soft water (LC_{50} 0.231 mg L^{-1}) (Villar *et al.* 2000).

According to published LC_{50} values adjusted to a total hardness of 50 mg L^{-1} as $CaCO_3$ for different Holartic freshwater fish species (USEPA 2000), the adjusted sensitivity for *O. bonariensis* to Cd(II) (mean acute 96 hr LC_{50}= 0.013 mg L^{-1}) is one order of magnitude above the most sensitive species (*Salmo truta* mean acute LC_{50}= 0.0016 mg L^{-1}), equivalent to *Pimephales promelas* and over three orders of magnitude below the least sensitive reported fish species (*Oreochromis mossambica* mean acute LC_{50}= 11,86 mg L^{-1}). When compared to the saltwater Atherinidaes *Menidia menidia* and *O. regia*, the studied species shows a sensitivity from two to three orders of magnitude higher (USEPA 2000, Silva *et al.* 2001).

We can conclude that most of the studied factors affect, under acute exposure, the sensitivity of *O. bonariensis* to Cr(VI), Cu(II) and Cd(II). Hence, variations in sensitivity between natural populations and the physicochemical characteristics of the water bodies should be carefully considered in risk assessment studies for the species in its natural environments.

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